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STUDIES ON ROPINESS IN CULTURES OF *STREPTOCOCCUS LACTIS*

By B. W. Hammer

The production of a ropy condition in milk is a character occasionally shown by the organisms of the *S. lactis* group. This character is, however, one that is rather inconstant and its gain or loss or a variation in its extent is frequently noted in the laboratory in cultures that are very probably pure. In starters also, where the *S. lactis* type of organisms makes up the largest percent of the flora, the development of ropiness occasionally occurs and when ropy starters are carried thru a number of transfers this ropiness sometimes disappears. The change from ropy to non-ropy cultures of *S. lactis* and the reverse have recently been studied by plating out one of the types, picking colonies and looking for the other among the cultures developing; the results obtained are herein reported.

HISTORICAL

Organisms of the general *S. lactis* type have been found to be the cause of ropiness in milk by various investigators. Many of the results reported have been reviewed by Buchanan and Hammer[1]. Gorini[2] in a recent summary of his work states that, "many lactic acid forming organisms are capable of inducing ropiness in milk only during the early phases of incubation," and that, "the reason why many investigators disagree as to the constancy of this rope-producing property in many of the lactic acid bacteria is to be searched for in its transitory manifestation."

METHODS

Whey agar was used in all plating work on pure cultures of *S. lactis*, because of the good growth made on this medium. The plates were poured by shaking up in a water blank a small amount of the culture picked up on a sterile wire and using two very different amounts of the dilution water, so that one of the plates could be expected to give a good distribution of

1. Buchanan, R. E. and Hammer, B. W. Slimy and Ropy Milk, Res. Bul., Ia. Agr. Exp. Sta. 22, Jl., 1915.
2. Gorini, Costantino. Studies on the Biology of Lactic Acid Bacteria. Jr. of Bact. 7: 271. Mr. 1922.

colonies. The plates were held at room temperature for two or three days and colonies then picked into litmus milk. The milk was also held at room temperature for two or three days, at which time coagulation had occurred, and then examined for ropiness by introducing and withdrawing a wire loop that had been sterilized; a ropy condition could also in general be detected by slightly agitating the milk, but this procedure was always checked with the loop method. In picking from plates, contiguous colonies were taken with the idea of getting more representative results than would have been secured if colonies that were not contiguous had been taken.

The volatile acid determinations on fermented milk were made according to the method previously followed by this station[3], as were also the determinations of the type of lactic acid[4]

RESULTS OBTAINED

The first ropy organisms studied were secured from ropy cream sent in from an Iowa creamery (W) by one of the extension men doing cream scoring work. When collected, the cream was ropy but sweet. It was sent by special delivery without ice and reached the laboratory the next morning, at which time it showed a very ropy condition and an acidity of .59 percent. The cream was plated at once on whey agar and after two days' incubation at room temperature, the colonies developing indicated a bacterial content of 740,000,000 per c. c. From one of the plates 37 contiguous colonies were picked into tubes of litmus milk; 28 of the resulting cultures were very evidently of the *S. lactis* type and, of these, 11 were very ropy while 17 were not. The ropy cultures were easily separated from the non-ropy because if cultures were at all ropy, they were decidedly so. The presence of a ropy condition in the cream before it had soured suggested non-acid organisms as a cause of the ropiness and accordingly it seemed that possibly the ropy cultures of *S. lactis* were impure. Two of these ropy cultures, *1a* and *1b*, were studied for purity by plating on whey agar and picking colonies into litmus milk. From the plates poured

3. B. W. Hammer and D. E. Bailey. The Volatile Acid Production of Starters and of Organisms Isolated from Them. Res. Bul. Ia. Agr. Exp. Sta. 55, S. 1919.
Also B. W. Hammer. Volatile Acid Production of *S. lacticus* and the Organisms Associated with it in Starters. Res. Bul. Ia. Agr. Exp. Sta. 63, O. 1920.
Also W. A. Cordes and B. W. Hammer. The Relation between the Volatile and Total Acidity in Starters and in Cultures of *S. lacticus*. Res. Bul. Ia. Agr. Exp. Sta. 66, Jl. 1921.
4. B. W. Hammer. The Type of Lactic Acid Produced by Starters and by Organisms Isolated from them. Res. Bul. Ia. Agr. Exp. Sta. 65, N. 1920.

with culture *1a*, 38 colonies were picked, 11 of which proved to be *S. lactis* and definitely ropy, and when one of these was plated and colonies picked, all of the 41 were ropy cultures of the *S. lactis* type.

Culture *1b* was studied more completely than culture *1a*, and while in a considerable number of instances all of the cultures secured from a plate poured with a ropy culture were ropy, it was not unusual to find among the basket of cultures secured from plates poured from a ropy culture one or two (and in one instance four) which were definitely non-ropy. When non-ropy cultures were plated it was usual to find that all of the cultures developing from the colonies picked were non-ropy in character; in only one instance out of 16 were ropy cultures secured by plating a non-ropy culture. While with certain organisms it is sometimes difficult to determine whether or not a culture is ropy, in the cultures studied the ropiness, when present, was very pronounced and a separation into ropy and non-ropy types was easily made; moreover, the facts that so many non-ropy cultures were plated and colonies picked without finding ropiness in the resulting cultures, and that the ropy cultures when plated yielded largely ropy cultures, suggest that the separation into ropy and non-ropy cultures was accurate.

The results secured in the study of culture *1b* are given in detail in table I. Ropy cultures were plated out 19 times and the colonies picked yielded cultures that showed varying percentages that were ropy; in ten instances 100 percent of the colonies picked from a plate gave ropy cultures, in one instance 98 percent gave ropy cultures, in four instances 97 percent gave ropy cultures, in two instances 95 percent gave ropy cultures, in one instance 93 percent gave ropy cultures, and in one instance 89 percent gave ropy cultures.

In 9 times out of the 19 when ropy cultures were plated, one or more non-ropy cultures were found among those picked. The percentage of the total number of cultures picked that were non-ropy varied in these 9 cases, being 2 percent in one instance, 3 percent in four, 5 percent in two, 7 percent in one and 11 percent in one. From table I, it is evident that the production of non-ropy cultures from ropy cultures occurred mainly in the early part of the plating work.

In the 16 platings of non-ropy cultures all of the colonies picked gave non-ropy cultures in 15 instances, while in one the percentage of the cultures that were non-ropy was 95 percent, leaving 5 percent that were ropy. It is accordingly evident that it was much more unusual to get ropy cultures from platings of cultures that were non-ropy than to get non-ropy cultures from platings of cultures that were ropy.

The securing of both ropy and non-ropy cultures of *S. lactis* from plates poured from a culture that was supposedly pure suggested a microscopic study of the two types. Such an examination was frequently carried out. Both the ropy and non-ropy cultures regularly showed large numbers of spherical or slightly elongated cells arranged in pairs, and an occasional clump apparently made up of pairs of cells irregularly packed together. Some of the clumps were small and contained only a few pairs of cells, while others seemed to contain hundreds of pairs grouped together. Altho the clumps were quite readily

TABLE I. ROPY AND NON-ROPY CULTURES SECURED FROM PLATINGS OF *S. LACTIS* ORGANISMS.

Culture Plated	Cultures Picked from One Plate						
		Ropy			Not Ropy		
	Total No.	No.	Per-cent	Cultures Saved for Plating	No.	Per-cent	Cultures Saved for Plating
1 b-ropy*	34	34	100	r-1 b 1	0	0	
r-1 b 1	40	39	97	r-1 b 2	1	3	nr-1 b 2
r-1 b 2	39	37	95	r-1 b 3 and r-1 b 4	2	5	nr-1 b 3 and nr-1 b 4
nr-1 b 2	40	0	0		40	100	
r-1 b 3	40	39	97	r-1 b 5	1	3	nr-1 b 5
r-1 b 4	41	40	98	r-1 b 6	1	2	nr-1 b 6
nr-1 b 3	33	0	0		33	100	
nr-1 b 4	38	0	0		38	100	
r-1 b 5	31	30	97	r-1 b 7	1	3	nr-1 b 7
r-1 b 6	27	25	93	r-1 b 8	2	7	nr-1 b 8
nr-1 b 5	36	0	0		36	100	
nr-1 b 6	32	0	0		32	100	
r-1 b 7	37	36	97	r-1 b 9	1	3	nr-1 b 9
r-1 b 8	43	41	95	r-1 b 10	2	5	nr-1 b 10
nr-1 b 7	31	0	0		31	100	
nr-1 b 8	33	0	0		33	100	
r-1 b 9	34	34	100	r-1 b 11 and r-1 b 11a	0	0	
r-1 b 10	37	33	89	r-1 b 12	4	11	nr-1 b 12
nr-1 b 9	36	0	0		36	100	
nr-1 b 10	37	0	0		37	100	
r-1 b 11	37	37	100		0	0	
r-1 b 11a	31	31	100	r-1 b 13	0	0	
r-1 b 12	40	40	100		0	0	
nr-1 b 12	39	2	5	r-1 b 14	37	95	nr-1 b 14
r-1 b 13	37	37	100		0	0	
r-1 b 14	35	35	100	r-1 b 15	0	0	
nr-1 b 14	38	0	0		38	100	nr-1 b 16
r-1 b 15	36	36	100	r-1 b 17	0	0	
nr-1 b 16	37	0	0		37	100	nr-1 b 18
r-1 b 17	36	36	100	r-1 b 19	0	0	
nr-1 b 18	37	0	0		37	100	nr-1 b 20
r-1 b 19	36	36	100		0	0	
nr-1 b 20	36	0	0		36	100	nr-1 b 21
nr-1 b 21	34	0	0		34	100	nr-1 b 22
nr-1 b 22	33	0	0		33	100	

*Original culture.

TABLE II. COMPARISON OF ACIDITIES (CALC. AS LACTIC ACID) OF ROPY AND NON-ROPY CULTURES.

Incubation was seven days at room temperature.

Ropy cultures		Non-ropy cultures	
Culture number	Percent acid	Culture number	Percent acid
r-1 b 3	1.071	nr-1 b 3	1.089
r-1 b 4	1.098	nr-1 b 4	1.089
r-1 b 5	1.044	nr-1 b 5	1.035
r-1 b 6	1.044	nr-1 b 6	.945
r-1 b 7	1.089	nr-1 b 7	1.062
r-1 b 8	1.089	nr-1 b 8	1.080
r-1 b 9	1.071	nr-1 b 9	1.062
r-1 b 10	1.044	nr-1 b 10	1.026

detected in any preparation, it was not unusual to look thru a number of fields before finding them. It was clearly evident that the ropy and non-ropy cultures could not be told apart by a microscopic examination; in a number of instances where this was attempted (the slides being prepared by someone other than the person examining them) on the basis of considering that the ropy cultures contained the larger number of clumps, the results were entirely unsuccessful.

The acidities (calc. as lactic acid) of the ropy and non-ropy cultures were compared in a number of instances as it was thought that a higher acidity in one or the other type would indicate an increased growth. The results are given in table II; the original acidity of the milk was not deducted from the values given for the different cultures. Altho the data presented show that in seven of the eight comparisons the ropy culture had a slightly higher acidity than the corresponding non-ropy culture, the differences are too small to justify any conclusions, since they often represent only 0.1 c. c. of the alkali solution used and moreover, some of the non-ropy cultures produced acidities higher than some of those produced by the ropy cultures.

In order to have additional data of importance from the standpoint of the classification of the organisms worked with, the type of lactic acid produced was studied with one ropy and one non-ropy culture, and the volatile acid production with two cultures of each type. The results are given in table III and show that the organisms produce *d* lactic acid (since the zinc lactate was of the *l* type) and a low volatile acidity as do typical *S. lactis* cultures.

The sample of cream originally studied was received at the creamery (W) on June 7, at which time the producer was given directions as to the care of cream by the extension man collecting the sample, and after this no more ropy cream was brought

TABLE III. VOLATILE ACIDITY AND TYPE OF LACTIC ACID PRODUCED BY ROPY AND NON-ROPY CULTURES.

Type of Culture	Volatile acid* production	Results obtained on $(CH_3\text{-}CHOH\text{-}COO)_2Zn$			
		Moisture content			Specific rotation
		A	B	Average	
Ropy	6.7				
Ropy	9.6	12.83	12.84	12.835	*l* 7.25
Non-ropy	7.8	12.94	13.07	13.005	*l* 7.74
Non-ropy	9.4				

*The figures given represent the c.c. of n/10 alkali required for the neutralization of the first liter of distillate obtained when a 250 gram portion of milk fermented by the organism was distilled with steam after the addition of 15 c.c. of approximately n/1 H_2SO_4.

in by the producer in question. On October 8 another sample was secured from the producer with the idea of again looking for the ropy type of *S. lactis*. The sample which was sweet and not ropy when collected was sent to the laboratory without ice, where it was received in a sour and slightly ropy condition. It was plated on whey agar and after four days incubation at room temperature contiguous colonies were picked into litmus milk; of the 27 *S. lactis* cultures secured, five were typically ropy, and when examined microscopically showed the same types of cells and clumps that were shown by the cultures secured four months previously. These findings show how, in certain instances at least, contamination on producing farms with a definite type of organism apparently persists for considerable periods and also how efficient are proper methods of caring for cream in preventing certain types of fermentations, even when the organisms capable of producing these fermentations are in all probability present.

Ropy cultures were secured from a second sample of ropy sour cream sent in from an Iowa creamery (F) by one of the extension men scoring cream there. The sample was sent with ice and on arrival at the laboratory showed a very ropy condition. It was plated on whey agar and after two days incubation at room temperature colonies were picked into litmus milk. Of the 32 cultures of the *S. lactis* type that were secured 12 were typically ropy and 20 were not; of the 20 non-ropy cultures two produced a burnt or caramel flavor in milk *(S. lactis* var. *maltigenae*[5]*)* and this indicates that the non-ropy cultures were not all of one type. Two of the ropy cultures, *2a* and *2b*, were run thru a number of platings with the idea of de-

5. B. W. Hammer and W. A. Cordes. Burnt or Caramel Flavor of Dairy Products. Res. Bul. Ia. Agr. Exp. Sta. 68, Jl. 1921.

TABLE IV. ROPY AND NON-ROPY CULTURES SECURED FROM PLATINGS OF *S. LACTIS* ORGANISMS.

Culture Plated	Cultures Picked from One Plate						
		Ropy			Not Ropy		
	Total No.	No.	Per-cent	Cultures Saved for Plating	No.	Per-cent	Cultures Saved for Plating
2a-ropy*	33	33	100	r-2 a 1	0	0	
r-2 a 1	36	36	100	r-2 a 2	0	0	
r-2 a 2	35	34	97	r-2 a 3	1	3	
r-2 a 3	39	39	100	r-2 a 4	0	0	
r-2 a 4	38	37	97	r-2 a 5	1	3	
r-2 a 5	38	38	100		0	0	nr-2 a 5
nr-2 a 5	40	0	0		40	100	

*Original culture.

termining whether or not the colonies picked into litmus milk from a plate poured with a ropy culture would all be ropy. The results are given in tables IV and V and are presented in the same general way as those given in table I.

Table IV shows that in the six instances in which ropy cultures were plated and colonies picked only ropy cultures were secured in four while in each of two instances one non-ropy culture (equivalent to 3 percent) was secured. In the only instance in which a non-ropy culture was plated, the cultures secured by picking colonies were all non-ropy. From table V it will be seen that ropy cultures were plated in 11 instances and

TABLE V. ROPY AND NON-ROPY CULTURES SECURED FROM PLATINGS OF *S. LACTIS* ORGANISMS.

Culture Plated	Cultures Picked from One Plate						
		Ropy			Not Ropy		
	Total No.	No.	Per-cent	Cultures Saved for Plating	No.	Per-cent	Cultures Saved for Plating
2b-ropy*	35	34	97	r-2 b 1	1	3	nr-2 b 1
r-2 b 1	35	34	97	r-2 b 2	1	3	nr-2 b 2
nr-2 b 1	38	0	0		38	100	
r-2 b 2	37	37	100	r-2 b 3	0	0	
nr-2 b 2	38	0	0		38	100	
r-2 b 3	38	38	100	r-2 b 4	0	0	
r-2 b 4	39	38	97	r-2 b 5	1	3	nr-2 b 5
r-2 b 5	38	38	100	r-2 b 6	0	0	
nr-2 b 5	35	0	0		35	100	
r-2 b 6	35	32	91	r-2 b 7	3	9	nr-2 b 7
r-2 b 7	33	27	82	r-2 b 8	6	18	nr-2 b 8
nr-2 b 7	32	0	0		32	100	
r-2 b 8	28	26	93	r-2 b 9	2	7	nr-2 b 9
nr-2 b 8	37	0	0		37	100	
r-2 b 9	26	26	100	r-2 b 10	0	0	
nr-2 b 9	33	0	0		33	100	
r-2 b 10	34	34	100		0	0	

*Original culture.

that in five of these all of the cultures secured from the colonies picked were ropy; in three instances the cultures that were ropy were equal to 97 percent, in one instance to 93 percent, in one instance to 91 percent and in one instance to 82 percent. In the six instances in which non-ropy cultures were plated all of the cultures secured from the colonies picked were non-ropy.

The data presented in table IV and V agree in general with those given in table I in that it was not at all unusual to secure some non-ropy cultures when a ropy culture was plated and colonies picked into litmus milk. From both sets of results also it seems that when non-ropy cultures are plated and colonies picked ropy cultures are not likely to be secured. With the results given in tables IV and V, as with those given in table I, the accuracy of the separation into ropy and non-ropy cultures is evident from the results secured in plating the two types and picking colonies.

Microscopic examinations were made of the ropy and non-ropy cultures and both regularly showed many spherical or slightly elongated cells arranged in pairs with an occasional clump made up of the pairs of cells irregularly packed together, exactly as did the cultures obtained from the cream from creamery W. It was impossible to see any definite difference between the ropy and non-ropy cultures in microscopic preparations made from them.

The types of lactic acid and volatile acid production were checked up to see if the organisms studied agreed in these respects with the usual *S. lactis* cultures. The data secured are given in table VI and show a low volatile acidity and a zinc lactate of the *l* type which corresponds to lactic acid of the *d* type.

In the study of ropy *S. lactis* cultures from the two sources already considered the results presented show that it was not unusual to get non-ropy cultures when ropy cultures were plated

TABLE VI. VOLATILE ACIDITY AND TYPE OF LACTIC ACID PRODUCED BY ROPY AND NON-ROPY CULTURES.

Type of Culture	Volatile acid* production	Results obtained on $(CH_3\text{-}CHOH\text{-}COO)_2Zn$			
		Moisture content			Specific rotation
		A	B	Average	
Ropy	11.3	12.79	12.72	12.755	*l* 7.75
Ropy	8.9				
Ropy	11.4				
Ropy	10.6				
Non-ropy	8.9	13.13	13.15	13.14	*l* 7.62

*See table III.

on whey agar and colonies picked into litmus milk. A third series of results was secured in which, in the trials made, it was impossible to secure non-ropy cultures by plating out ropy ones and picking colonies. The original ropy cultures used in this series were isolated from ropy sour cream submitted from the testing laboratory of the Iowa state college creamery. The cream was plated on whey agar and after incubation, contiguous colonies picked into litmus milk; of the 16 *S. lactis* cultures secured nine were decidely ropy while seven were not. Two of the original ropy cultures were selected and one followed thru three sets of platings and the other thru four, using the same general procedure of selecting a ropy culture from each set of cultures as was employed in the work reported in table I; from the seven plates 211 cultures were secured and all of them were decidedly ropy. While these results do not show that it was impossible to secure non-ropy cultures from ropy cultures of this strain, since the number of cultures picked was not very large, they suggest this, and in addition definitely indicate that non-ropy cultures from ropy ones were much less common with the *S. lactis* cultures secured from the third source than with those secured from the first two sources. In this connection it is worthy of note that microscopic examination of ropy cultures from the third source showed the organisms to be spherical or slightly elongated cells, regularly arranged in pairs and scattered quite uniformly thruout the milk. There was no tendency to chain or clump formation but the organisms did seem to be present in larger numbers than is the case with the ordinary cultures of *S. lactis*.

The data presented in tables I, IV and V are summarized in table VII with the idea of comparing the results secured with the cultures obtained from the two sources. From this table it is evident that of the 36 ropy cultures plated out 17 or 47.2 percent yielded one or more non-ropy cultures when colonies were picked into litmus milk. The percentages varied from 33.3 percent for the data given in table IV to 54.5 percent for that given in table V. Of the 1,288 colonies picked from the plates poured with the 36 ropy cultures, 31 or 2.4 percent gave non-ropy cultures; the percentages varied from .9 percent for the data in table IV to 3.7 percent for the data in table V. The general agreement of the data given in tables IV and V, which deal with organisms isolated from the same source, with that given in table I is rather striking especially since these results are quite different than those secured with the organisms from a third source. An investigation showed that the two creameries (W and F) from which the samples of ropy cream were secured were only about 11 miles apart, so it is possible that contamination may have been from the same source.

TABLE VII. SUMMARY OF TABLES I, IV AND V SHOWING ROPY AND NON-ROPY CULTURES SECURED FROM PLATINGS OF ROPY CULTURES.

Source of data	Ropy culture plated	Total colonies picked	Nos. of platings yielding all ropy cultures	Percent of platings yielding all ropy cultures	Nos. of platings yielding some non-ropy cultures	Percent of platings yielding some non-ropy cultures	Total non-ropy cultures secured	Percent of total cultures that were non-ropy
Table I	19	691	10	52.6	9	47.4	15	2.2
Table IV	6	219	4	66.7	2	33.3	2	.9
Table V	11	378	5	45.5	6	54.5	14	3.7
Combined results	36	1,288	19	52.8	17	47.2	31	2.4

DISCUSSION OF RESULTS

From the results presented it is evident that when ropy cultures from two samples of ropy cream were plated and colonies picked non-ropy cultures were sometimes secured; in only one instance out of the 23 (16 in table I, one in table IV and six in table V) in which a non-ropy culture secured from a plating of a ropy culture was plated were ropy cultures secured. This change from a ropy to a non-ropy culture and the reverse show the variability of the lactic acid organisms worked with from the standpoint of the ropy character. The same variation may not obtain with other ropy cultures of lactics and in a small amount of work done with ropy *S. lactis* cultures from a third source the production of ropiness by cultures picked from a plate poured with a ropy culture was constant. The cultures that frequently yielded non-ropy cultures when a ropy culture was plated out and colonies picked had a peculiar clumped arrangement of some of the pairs of cells which was not the case with the ropy cultures that did not yield non-ropy cultures on plating.

The presence in a ropy culture of certain cells which alone would give a non-ropy culture makes possible a variation with respect to this character and the same is true of a non-ropy culture containing certain cells which would give a ropy culture. The elimination of one type of organism from a mixture of two by a series of transfers in some medium is sometimes easy and sometimes difficult and in this connection it is worthy of note that transfers made to litmus milk from cultures that were ropy always gave ropiness, while transfers from non-ropy cultures were always non-ropy. The presence, however, of cells that are different with respect to the production of ropiness

than the majority of the cells in a culture certainly suggests that minute contamination (as many contaminations must be under practical conditions) from a ropy culture might not always give the same results with respect to the ropy character and also that contamination from a non-ropy *S. lactis* culture might sometimes yield a ropy strain. The method of plating out and picking colonies shows the sudden variations in the cells of a culture. By a series of transfers these variations might never be noticed or if they were noticed there would probably be a gradual change as one type of organism was replaced by another; e. g. as non-ropy cells began to increase it would be expected that the ropiness would gradually decrease instead of disappearing suddenly.

Among the various causes of ropiness in cultures of microorganisms, the presence of large numbers of cells is the most probable explanation of the ropiness in the cultures studied. Both the ropy and non-ropy cultures showed large numbers of cells and it seems that the development of ropiness depends on a comparatively small additional number of cells, the extra number being too small to detect microscopically. This may be due to a shifting of the relationship between the number of cells and the acid development so that in certain cultures the number of cells present at the time the limiting acidity is reached is larger than with other cultures.

It is reasonable to suppose that variations occur in other characters in just the same way that they occur in the ropy character, and that accordingly organisms differing from each other in various ways might come from the same original cell. The development of a non-ropy culture from a ropy one is undoubtedly a comparatively simple change and it would be expected that more complex variations would occur less frequently than the variation involving ropiness. If variations occur, however, their frequency is of only minor importance from the standpoint of accounting for the development of different types of organisms. In this connection the importance of contributing factors should also be taken into consideration. There seems to be no advantage to the organisms in the development of a ropy or non-ropy character while other types of variations, such as the development of the ability to ferment a certain material, might be an advantage. The presence of this material in a medium in which the organism, unable to effect the fermentation, is growing might be a contributing factor in tending to make the culture vary with respect to this fermentation and such a contributing factor would be expected to counter-balance the anticipated infrequency of certain variations (as compared to variations in ropiness) due to the type of change involved.

The results presented along the line of the development of non-ropy cultures from ropy ones and the reverse make it seem certain that, as is maintained by many investigators, the ropy character is valueless from the standpoint of classification. If ropy or non-ropy cultures can be secured from the opposite type, although only infrequently, the character is not constant enough to be considered for the establishment of species or even types.

SUMMARY

In plating out ropy cultures of *S. lactis* on whey agar and picking colonies into litmus milk it was found that non-ropy cultures were sometimes secured. Of 36 ropy cultures from two sources that were plated, 17 or 47.2 percent yielded one or more non-ropy cultures among those developing from the colonies inoculated. The 1,288 colonies picked from plates poured with ropy cultures yielded 31 or 2.4 percent cultures that were non-ropy. In plating out 23 non-ropy cultures from the two sources ropy cultures were secured in only one instance, and then only to the extent of 5 percent of the colonies picked.

The cultures secured from plates poured with ropy cultures from a third source yielded in a small number of trials ropy cultures only. The organisms from this source differed from those from the first two sources in that they failed to show clumps made up of pairs of cells as did the other organisms.

Sudden variations in cells can be detected much better by plating and picking colonies than by making a series of transfers in some medium.

The sudden variations that occur in the ropy character of certain *S. lactis* cultures suggest that sudden variations may also occur with other types of organisms.

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